Conference Paper

The influence of algal food preparation on its nutritional efficiency for *Artemia salina* L. larvae

*P. Sorgeloos**

State University Ghent, Laboratorium voor biologisch onderzoek van milieuverontreiniging, J. Plateaustraat 22, B — 9000, Ghent (Belgium)

Feeding live food to aquatic invertebrates is a time consuming and very often, if not always a troublesome business for those involved in culturing problems.

It has already been shown (although the number of papers on the matter is scarce), that live food such as bacteria or algae, can be stocked for future use by freezing or lyophylization.

During experiments on the growth rate of larvae of the brine shrimp, *Artemia salina,* we have tested several preparation methods of the same algal food (*Dunaliella spec.*): living cells, suspensions homogenized by Ultrason, algal suspensions frozen at —25 °C and suspensions slowly evaporated to dryness, followed by resuspension and homogenization.

Preliminary results seem to indicate that the three ways of preparing the food, apparently do not influence drastically the nutritional efficiency of the algae.

The dried freshwater algae, *Scenedesmus* and *Spirulina* also can be used as food for the brine shrimp.

Adequate techniques for preparation of large food stocks from algae certainly greatly facilitate the culturing of a number of invertebrate larvae.

INTRODUCTION

Several studies have been published on the nutritional value of different unicellular algae as food for the brine shrimp *Artemia salina*[1—3].

Many algal species proved to constitute an excellent food for different life stages of this crustacean.

The algae have always been fed immediately after harvesting of the culture, they were mostly centrifuged and resuspend in fresh sea water to prevent further growth.

During a series of preliminary experiments using several algae, it soon appeared that it was very difficult to keep the cell concentrations of the harvested algae constant during the feeding experiments[4]. The algal density increases (division of the algae due to storage of photo-energy) or a part of the algal population dies (possibly by injury due to manipulations or osmotic shock caused by the change in the medium).

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Another difficulty in assessing the nutritional value of different algal species is the necessity to harvest them always during the exponentional growth phase of the culture.

Since the *Artemia* larvae have to be fed daily this implies that algal cultures in the logaritmic growth phase are always available. The only solution for this problem is to use a rather complex and expensive chemostatic or turbidostatic apparatus.

Most of these problems would be avoided if the algae, after being harvested during their exponential growth phase, could be stored in one way or another without loss of their nutritional value.

Although the number of papers on this subject is scarce, it has already been shown that live algal food can be stored. Brown[5] proved that freezing after the addition of some protectants was possible and Hidu and Ukeless[6] fed *Mercenaria* larvae with freeze-dried algae.

The present study deals with comparative experiments about the preparation and storage of two algae (the flagellate *Dunaliella* and the chlorococcal *Scenedesmus*) as food for *Artemia* larvae.

METHODS

Culturing chamber for Artemia

The larvae of *Artemia* have to be cultured in non-disturbed or only moderately air-bubbled media. Dutrieu[7] and von Hentig[8] report indeed a decrease in growth in heavily disturbed waters.

During the culture experiments it is of crucial importance that the food particles are kept in suspension and the water well oxygenated.

We found a solution to these problems with a most convenient »*Artemia* culture cylinder« (Fig. 1). The apparatus simply consists of a glass cylinder B (diameter 5 cm, height 50 cm) ending in a long narrow funnel which is connected to a lateral tube A parallel to the cylinder, and connected to an air-pump. Every 30 minutes the pump is switched on for one minute, bubbling air at a rate of approximately one bubble a second.

Both food particles and larvae are evenly suspended in the water column and the oxygenation of the water definitely meets the needs of the nauplii.

Cultivation procedure

Artemia cysts are hatched and the nauplii separated from the hatching debris according to the recently described procedures[9, 10]. A total of 250 larvae are counted with a special larvae counter[11] and transferred to the culturing cylinders, each containing 500 ml artificial sea water (formula of Dietrich and Kalle[12]). The nauplii were cultured in the following conditions: temperature 28 °C[13]; salinity 35‰[2]; feeding twice daily; quantity of food: 15.10^6 cells of a marine *Dunaliella spec.* Culturing of the algae was done following a technique that will be described elsewhere[14].

Four different preparations of the same algal food were tested, each experiment being run in triplicate:

A: living algal cells
B: algal suspension homogenized with ultrason
C: algal suspension frozen at —25 °C
D: algal suspension slowly evaporated to dryness, resuspended and homogenized

The algae were cultured, harvested and treated in the following way: two one liter flasks were inoculated with the same quantity of *Dunaliella* cells and cultured until a density of 6.10^6 algae per ml was reached. To be sure of a constant cell density

in each flask, the two cultures were finally introduced into a large beaker, the suspension thoroughly mixed and redistributed among the flasks. The cell density in the first flask was kept constant by checking the number of cells daily and diluting it with fresh medium.

The algae of 3 × 100 ml culture were harvested by centrifugation and the yield resuspended in 100 ml sea water, except for the last batch which was resuspended in 100 ml distilled water. In the first algal suspension in sea water, the cells were broken by homogenizing them for 10 minutes by ultrason (Branson Sonifier B-12, energy of 80 Watts — Molkenboer[15]). The second sea water algal suspension was frozen for a week at —25° C, which made all the cells burst (as could be observed under the microscope). The freshwater algal suspension was finally slowly evaporated to dryness, avoiding burning of the cells. The residue was resuspended and homogenized in 100 ml sea water with ultrason at a low energy.

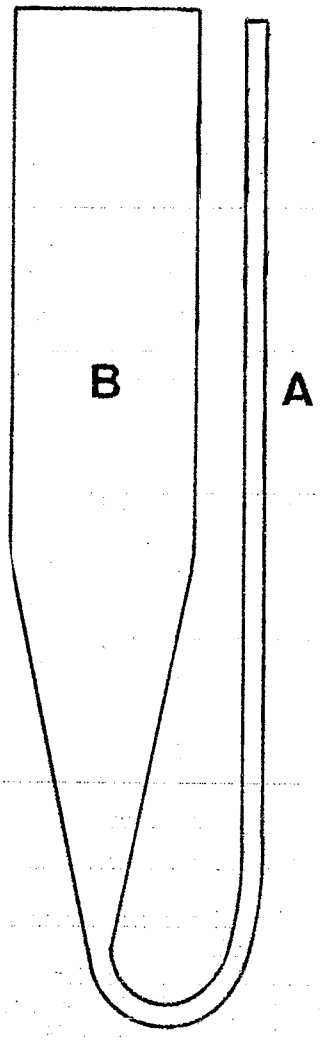

Fig. 1. *Artemia* culture cylinder
A. Lateral tube
B. Culture cylinder

To prevent bacterial growth, all the treated algal suspensions (stocks B, C, and D) were kept at 0 °C. At each feeding time 10 ml of the live algal culture were harvested, centrifuged, and the algae resuspended in 10 ml sea water (stock A).

The preparation of the four different food media has been chosen as to contain the same density of *Dunaliella* in each suspension.

The *Artemia* cultures were fed twice daily with 2.5 ml of the respective stock solutions, for a period of four days. Fifty individuals of each culture were deep-frozen at the end of the experiment; the rest of the larvae were fixed by adding 2 ml lugol solution to each cylinder and the percent survival was determined.

The frozen larvae were lateron thawed and transferred to glass slides. The total length (from the anterior margin of the head in front of the ocellus to the base of the caudal furca[16]), was measured using a projection system. The average length, standard deviation of the mean and coefficient of variation were computed.

TABLE I

Survival and growth data of the Artemia *larvae in the series A, B, C, and D.*

Method of food preparation	Percent survival	Average length (in micron)	Standard deviation of the mean (in micron)	Coefficient of variation (in micron)
A — Living algal cells	98	2573	251	9,8
	96	2568	249	9,7
	96	2541	238	9,4
B — Algal suspension homogenized with ultrason	89	2180	201	9,2
	87	2149	151	7,0
	90	2168	194	8,9
C — Algal suspension frozen at —25 °C	97	2451	181	7,4
	97	2448	190	7,8
	97	2460	208	8,5
D — Algal suspension slowly evaporated to dryness and resuspended	97	2346	187	8,0
	95	2320	180	7,8
	97	2331	192	8,2

RESULTS

Survival and growth data are summarized in Table I. From these data it is clear that:

1. The survival is high for all the experiments, being approximately 97% in A, C and D; and 89% in B.
2. The growth rates in A, C and D are slightly different from each other: series A being better than C, and C better than D.
   The slowest growth rate occurs in series B.
3. In all the series the standard deviation of the mean and the coefficient of variation are low.

DISCUSSION

The four different preparations of the same algal food do not result in a marked difference of their nutritional efficiency for *Artemia* larvae.

The smallest growth rate which was obtained with the Ultrason homogenized algae may be due to the extreme reduction of the particle dimensions to the level of cellular inclusions, *i. e.* only a part of the added food particles remains large enough to be taken up by the filtering setae of *Artemia,* which is an obligate particle-feeder[17].

The growth data indicate that the *Artemia* larvae from series A grew slightly faster than those in the other experiments. From this it should appear that living cells have the highest food-energy! It is, however, possible that in series A (living algal cells), the larvae could filter more cells, although they received exactly the same amount of algal cells as in the other series.

It is indeed known that due to the storage of photo-energy, algae can continue to divide even after the transfer from light into conditions of complete darkness.

It was however not possible for us to check this effect in the above experiment.

As there is only a negligible growth difference between series C and D, it is clear that during the evaporation and drying process there is no obvious loss of food energy as far as its conversion value is concerned.

The present data seem to indicate that stocking food algae by freezing or drying apparently do not influence drastically their nutritional efficiency. Homogenizing the algae by ultrason, however, possibly reduces the particle size to the critical filtering level.

These experiments clearly show that for feeding purposes the algae can be mass cultured, harvested during the exponential growth phase and stocked as desired for later use.

Bearing this in mind we tried to find out is easy to culture freshwater alge as for example the chlorococcal species *Scenedesmus* (Prokes and Zahradnik[18] and Soeder *et al.*[19]) would not be as good a food for brine shrimp larvae as marine unicellular flagellates. We therefore fed *Artemia* larvae in different paralleles with respectively living, frozen, and dried *Scenedesmus* cells.

The results were obvious: *Artemia* larvae are able to take up the algae but only the dried cells could be digested, the other cells were excreted without any digestion.

The masticating mouth-parts of the brine shrimp seem to be unable to break up the cell wall. This seems to be confirmed by Gibor[20], who found that the marine chlorococcal species *Stichococcus fragilis* could not be digested by *Artemia* due to the rigidity of the cell wall.

Prowse (in Costlow[21]), reported that the cellulose cell walls of Chlorophyceae are not permeable to the digestion enzymes of herbivorous fish. Possibly this might be the same for filter-feeders.

Several authors (quoted in a review of Soeder and Pabst[22], pp. 610—611), tested the efficiency of a *Scenedesmus* diet on vertebrates and found that the algae dried on a heated drum had the highest digestibility. Drying the cells on a heated drum breaks the cell walls much more efficiently than by freezing,

air-drying, vacuum drying or lyophilisation techniques. Although the *Artemia* larvae cultured on a *Scenedesmus* powder diet had the same growth rate as larvae fed with *Dunaliella,* only a part of the *Scenedesmus* food is metabolized.

The faeces are green colored due to the accumulation of the undigestible cell walls, whereas they are dark brown in the case of a *Dunaliella* feeding.

The food conversion efficiency is lower with *Scenedesmus* than with *Dunaliella.*

CONCLUSIONS

Artemia larvae can be cultured on a diet of dried or frozen algal cells. As it has been proven that cladocerans[23, 24], and copepods[25] can ingest inert particles, it is probable that as well freshwater as marine filter-feeders may be cultured on a diet of dried or in some cases frozen algal cells. This indeed solves a number of problems and undoubtedly opens a wide range of prospectives:

1. Culturing of particular organisms for toxicological, genetical, biochemical and other research can easily be done without the time consuming and often troublesome business of needing a continuous supply of living algae from a culture in the exponential growth phase.
2. In ecological research it is possible to keep the parameter food quality and/or quantity constant.
   D'Agostino and Provasoli[26] for example in their article »Effects of salinity and nutrients on mono- and diaxenic cultures of two strains of *Artemia*« could not draw pertinent conclusions since themselves had to admit that the observed effect might be mediated through the living algae.
   Provasoli (in Costlow[21]) even clearly says that the nutritional value of the algae may depend on the conditions to which the algae are subjected either during their culturing or during the feeding experiment. Moreover, experiments on the influence of light on the growth rate or reproduction rate of an invertebrate also require the use of a non-living food which may not be affected by the variable light parameter[27, 28].
3. As dried algal powders will soon be commercially available, it will be possible to develop mass-culturing techniques for filter-feeding herbivores[29].

NOTE

Just recently we received through Mrs. Clement from the »Institut Français de Pétrole« a small quantity of *Spirulina* cells, dried by lyophilisation.

Spirulina maxima is a blue green alga which is actually mass cultured in Mexico (1 ton/day, Feldheim[30]).

According to Clement and van Landeghem[31], the dried powder contains up to 70% proteins.

The first culture-data which we obtained with this type of food for *Artemia* nauplii have shown that the *Spirulina* cells are taken up and completely digested (faeces brown-black).

Larval growth rate is very good!

Further detailed experiments on the determination of the conversion efficiency of this food and the optimum growth rate of *Artemia* larvae on a *Spirulina* diet are in progress.

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IZVOD

Utjecaj načina pripremanja hrane iz algi na njihovu hranjivu vrijednost kod ličinke *Artemia salina* L.

P. Sorgeloos

Hranjenje morskih invertebrata živom hranom predstavlja vrlo često i gotovo uvijek dodatan posao što se tiče vremena za one koji su uključeni u probleme uzgoja. Već je ranije ustanovljeno (iako mali broj publikacija) da se živa hrana, kao što su bakterije ili alge, može spremiti za kasniju upotrebu smrzavanjem ili liofilizacijom. Ispitivano je nekoliko metoda pripremanja iste hrane od vrste *Dunaliella spec.* (žive stanice, homogenizirane suspenzije pomoću Ultrasona, suspenzije algi smrznutih kod —25 °C i suspenzije polagano isparavanih do suhoće koje su nakon toga bile resuspendirane i homogenicirane) u pokusima prirasta ličinka račića *Artemia salina.*

Preliminarni rezultati su pokazali da tri načina pripremanja hrane, očito, ne utječu drastično na prehrambenu vrijednost algi. Suhe slatkovodne alge *Scenedesmus* i *Spirulina* mogu se također upotrebljavati kao hrana. Odgovarajuće tehnike za pripremanje većih zaliha hrane od algi sigurno bi olakšale uzgoj izvjesnog broja ličinki invertebrata.